PATENT
Customer Number 22,852
Attorney Docket No. 1147-0142

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Reissue Application of: U.S. Patent No. 5,750,338	)	
Mark L. Collins et al.	)	Group Art Unit: 1655
Reissue Serial No.: 09/533,906	)	Examiner: D. Johannsen
Reissue Application Filed: March 8, 2000	)	
For: TARGET AND BACKGROUND CAPTURE METHODS WITH AMPLIFICATION FOR AFFINITY ASSAYS	)	

REISSUE LITIGATION BOX
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

DECLARATION OF ANDREW P. FEINBERG, M.D.

I, Andrew P. Feinberg, M.D., declare as follows:

1. I received my bachelors degree, M.D., and M.P.H. degrees from Johns Hopkins University in 1973, 1976, and 1981, respectively. I performed research fellowships at the University of California San Diego from 1977 to 1979 and at Johns Hopkins University School of Medicine from 1981 to 1983. During the latter period I invented the random priming procedure for labeling DNA. This work was published in *Analytical Biochemistry*, vol. 132, pp.

6-13 (1983), "A Technique for Radiolabeling DNA Restriction Endonuclease Fragments to High Specific Activity," by Andrew P. Feinberg and Bert Vogelstein. This article has been cited many thousands of times in the literature and I am intimately familiar with the techniques described therein.

2. My awards include general honors of Johns Hopkins University; Phi Beta Kappa; Delta Omega; Johns Hopkins University School of Medicine award for postdoctoral investigation; Fellow of the American College of Physicians; Member, American Society for Clinical Investigation; Member, Association of American Physicians; Institute for Scientific Information most-cited authors list; and Dean's Lecturer at Johns Hopkins University School of Medicine and the University of Kentucky School of Medicine.

3. I was an instructor in Medicine from 1979 to 1980, and Assistant Professor of Oncology and Medicine from 1983 to 1986, at Johns Hopkins University School of Medicine. I was an Assistant and Associate Professor of Internal Medicine and Human Genetics at the University of Michigan from 1986 to 1994 as well as a Howard Hughes Investigator at that institution. Since 1994, I have been King Fahd Professor of Molecular Medicine in the Institute of Genetic Medicine and the Department of Medicine at Johns Hopkins University School of Medicine, with joint appointments in Oncology and Molecular Biology & Genetics.

4. I am the author of over 100 articles, including 78 peer-reviewed original reports. Most of these publications are in "high impact" journals, including *Science*, *Nature*, *Cancer Research*, *Nature Genetics*, *Human Molecular Genetics*, *Journal of the National Cancer Institute*, *Nature Medicine*, *Proceedings of the National Academy of Sciences*, *Blood*, *American Journal of Human Genetics*, *Journal of Clinical Investigation*. I am considered an expert in gene technology and am an inventor on six patents awarded or pending. A copy of my current *curriculum vitae* is attached to this Declaration as Exhibit A.

5. I have reviewed the specification, drawings, and claims of U.S. Patent No. 5,750,338 ("the '338 patent). I understand that the specification of the '338 patent is identical to the specification of the above-referenced reissue application. I have also reviewed the contentions of Gen-Probe Incorporated ("Gen-Probe") that the specification of the '338 patent does not enable the non-specific amplification methods within the scope of claims 1-40 of the '338 patent, which I understand have been presented as claims 1-40 of the above-referenced reissue application.

6. In my opinion, Example 5 of the '338 patent, which addresses the use of random oligonucleotides to cause replication of target DNA, could have been successfully carried out as of December 21, 1987 by a person of ordinary skill in the art without undue experimentation.

7. It is also my opinion that Example 6 of the '338 patent, in which the Klenow fragment of DNA polymerase is added in appropriate buffer with random hexamer oligonucleotides to bring about non-specific double-stranded DNA synthesis, could have been successfully carried out as of December 21, 1987 by a person of ordinary skill in the art without undue experimentation.

8. It is also my opinion that other techniques known in the art would have enabled one skilled in the art to successfully carry out target nucleic acid amplification using methods other than Examples 5 and 6 as of December 21, 1987.

9. The use of random primers to achieve amplification of target nucleic acid is described in several places in the '338 patent, and particularly in Examples 5 and 6 and Figures 5 and 6.

10. The diagram in Figure 6, and the description referring to that Figure, is sufficient to allow one of ordinary skill in the art to perform amplification of a target polynucleotide using DNA polymerase and random primers. It correctly includes hybridization of primers to target

DNA, generation of a first complement target, DNA denaturation, a second round of DNA polymerase to generate a second complement to the target, and so forth.

11. My *Analytical Biochemistry* publication describing the use of random hexanucleotide primers for the generation of DNA product provides for denaturation conditions, annealing conditions, polymerization conditions, and quantification. It is clear from the description in my publication that there is enormous latitude in the reaction conditions that would permit successful DNA synthesis. While the purpose of that original publication was the generation of radioactive probes, and hence additional products were not necessary, it was clear that additional denaturation and polymerization would lead to additional product.

12. While we did not use repeated cycles of denaturation and renaturation to amplify the target, such an approach is clearly described in U.S. Patent No. 4,683,202 (July 28, 1987) ("the '202 patent"), which discloses the widely known PCR procedure. That procedure has two essential elements: cycles of denaturation, annealing, and renaturation, which are also described in the '338 patent, and also the use of specific primers to amplify a specific target sequence. The '202 patent also describes a variety of reaction conditions that would permit successful amplification of a target nucleic acid.

13. However, the use of specific primers is unnecessary in some embodiments of the invention of the '338 patent, for in those embodiments one is not trying to amplify a specific sequence over a background presence (sequence present on the same membrane, surface, or solution). Rather, the amplification step in those embodiments is meant to amplify the target sequence that has already been captured (separated from the background). The capture procedure itself is not dependent on amplification, but rather the many steps described in the patent prior to this step and described in Figures 1-3.

14. The '338 patent provides two examples that directly address the issue of amplification using random primers, Examples 5 and 6. Clearly, either example could be used as

an approach for amplifying the target DNA. Example 5 involves both DNA polymerase and RNA polymerase, as also described in Figure 5. Example 6 involves DNA polymerase, specifically the Klenow fragment. In Example 5, specific conditions are provided that are suitable for the use of both RNA polymerase and DNA polymerase. These include specific concentrations of buffer, pH, magnesium, and deoxynucleotides. I note in this regard that there is an obvious typographical error in the deoxynucleotide concentrations in Example 5. Thirty millimolar should obviously by 30 micromolar. It would otherwise be 200 times higher than the concentrations used in Example 5 and used conventionally in the art. The selection of appropriate reagent concentrations for this type of reaction was well within the capabilities of a person of ordinary skill in the art in December of 1987.

15. Moreover, it was well-understood by those skilled in the art that reaction conditions (such as concentration of primers, polymerases, and dNTPs, incubation times and temperatures, pH, and buffer components) could be varied while still achieving amplification of a target nucleic acid. Varying those conditions was considered to be routine by those skilled in the art.

16. As of December 21, 1987, a person of ordinary skill in the art could also have achieved non-specific amplification of a target polynucleotide by using the polymerase chain reaction ("PCR"). PCR was the most famous amplification method known as of December 21, 1987. By practicing PCR at conditions of low stringency, one could have easily obtained non-specific amplification of target polynucleotides. *See, e.g.*, Mullis et al., Specific Enzymatic Amplification of DNA *In Vitro*: The Polymerase Chain Reaction in Cold Spring Harbor Symposia on Qualitative Biology, Vol. LI, pgs. 263-273 (1986) (describing use of Klenow fragment to amplify globin sequences at 30° C yielding only 1% specific sequences at page 270); Saiki et al., Primer Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase, Science, Vol. 239, pgs. 489-491 (1988) (describing PCR with Klenow fragment at

37°C as resulting in non-specific extension of unrelated sequences under non-stringent hybridization conditions at page 490).

17. I am familiar with Gen-Probe's contention that the specification of the '338 patent does not enable non-specific amplification methods. I understand that in support of that contention, Gen-Probe has asserted that Example 6 of the '338 patent was never successfully practiced by the Patent Owner or its predecessor company, Gene-Trak Systems, Inc. ("Gene-Trak").

18. I have reviewed laboratory notebooks and periodic reports of the Gene-Trak scientists involved in this work. The material I reviewed does not affect my opinion that as of December 21, 1987, a person of ordinary skill in the art could have achieved amplification of a target nucleic acid using random primers without undue experimentation, in view of the disclosure of the '338 patent and the knowledge available to those skilled in the art.

19. I reviewed Gene-Trak laboratory notebook numbers 23, 63, 85 and 91 upon which Gen-Probe relies. The material I reviewed appears to reflect casual, exploratory work on using random primers to amplify nucleic acid. Moreover, the material I reviewed indicates that the vast majority of the experimental work using random primers reflected in Notebooks 63 and 91 was performed by an individual I am informed did not have a Doctor's degree, but instead had completed a Masters degree program just prior to joining Gene-Trak in the fall of 1987. I would not consider this individual to be a person of ordinary skill in the art as of December 21, 1987, particularly in light of the nature of the experimentation recorded in these notebooks. Further, I do not consider the level of experimentation undertaken by this individual even to rise to the level of "routine" experimentation.

20. My review of the experiments in Notebooks 63 and 91 indicates that there were numerous flaws in the experimental techniques. For example, I believe that the experimenter used different reaction volumes in different experiments, but did not appreciate the effect that

these volumes would have on concentrations of reactants. Further, some of the experiments demonstrate that little attention was paid to controlling all of the variables in the experiments, which would lead to wholly unreliable results.

21. In some of the temperature-cycling experiments, 87° C is reported as the temperature for denaturation. That temperature is too low to denature newly-synthesized double-stranded DNA, thereby preventing the newly-synthesized strands from serving as a template for future synthesis. Choosing that temperature was a major error that would not have been made by a person of ordinary skill in the art.

22. Notwithstanding the lack of a serious attempt to perform non-specific amplification using random primers and the numerous mistakes made by the Gene-Trak experimenter, Gene-Trak was still able to obtain amplification of a target nucleic acid using random primers and temperature cycling by November 1987. My review of laboratory notebook 63 indicates that ten cycles of denaturation, annealing, and synthesis led to the production of more target nucleic acid (measured by incorporation of radioactive NTPs) compared to a control that was not cycled.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

February 20, 2002

Andrew Feinberg, M.D.